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Research Article

Effectiveness of sengon ethyl acetate extract and bamboo liquid smoke as disinfectans

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ABSTRACT

The absence of environmental sanitation can influence the transmission of infectious diseases, exemplified as toilets tainted with dangerous microbes. Prevention typically employs disinfectants; nevertheless, their adverse effects might lead to skin and respiratory discomfort. Flavonoids, tannins, and saponins in sengon wood, phenol and acetic acid in bamboo liquid smoke have antibacterial properties and can serve as alternative materials. The study aimed to assess the efficacy of combining sengon wood extract and bamboo stem liquid smoke as a disinfectant. The experimental methodology employed in vitro testing through an inhibitory assay and in vivo testing utilizing a swab on the surface, assessing the effects pre- and post-swabbing. The in vitro results indicated that the most substantial inhibitory zone was observed with a 40:60 ratio for *Salmonella typhimurium* ATCC 25241, measuring 11.8 mm; for *Candida albicans* ATCC 10231, a 30:70 ratio yielded 5.33 mm; and for *Aspergillus flavus* ATCC 9643, a 30:70 ratio resulted in 5.5 mm. In vivo, swabs from the table yielded an average reduction of 92% in bacterial colonies and 73% in fungal colonies.The amalgamation of sengon twig wood extract and bamboo stem liquid smoke exhibits potential as a disinfectant.

Keywords: bamboo sticks, disinfectant, liquid smoke, sengon wood

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INTRODUCTION

Disinfectants serve to eradicate germs on the surfaces of inanimate items. Disinfectants often comprise glutaraldehyde and formaldehyde (Lululangi et al. 2020). Contact with disinfectants may result in adverse effects, including skin irritation, eye irritation, respiratory complications, and potential poisoning (Zulfikri & Khairina, 2020). The prolonged use of chemical disinfectants may have adverse effects. Chemical residues can lead to health issues (Broto et al. 2021). Consequently, it is imperative to identify alternatives for employing natural materials in the production of plant-based disinfectants. Natural substances suitable as raw materials for disinfectants encompass sengon plants and liquid smoke derived from bamboo stems.

Sengon (*Falcataria moluccana*) is a plant species indigenous to West Java, characterized by rapid growth, adaptability to several soil types, favorable silvicultural traits, and wood quality suitable for the panel and carpentry industries (Pragita et al. 2020). Secondary metabolites in sengon plants exhibit antibacterial capabilities (Rumidatul et al. 2021). The phytochemical analysis of sengon secondary metabolites indicates that sengon extract possesses antibacterial properties due to the presence of terpenoids, steroids, flavonoids, and phenolic compounds (Firdausia et al. 2021).

Liquid smoke can be generated by the bamboo pyrolysis process, occurring concurrently with charcoal production. Liquid smoke derived from pyrolyzed bamboo stems possesses elevated levels of acetic acid and phenol, exhibiting antibacterial effects (Pah et al. 2022). Phenol possesses antibacterial and antioxidant characteristics, which, when combined with organic acids, can effectively inhibit microbial development. The elevated phenol concentration in liquid smoke can serve as the primary component in disinfection formulations (Rosmainar et al. 2021).

A study is required to evaluate the efficacy of a mixture of natural disinfectants derived from secondary metabolites of sengon wood, extracted with ethyl acetate, and liquid smoke from bamboo stems on microbial growth. This study aims to assist the community in decreasing the reliance on chemical disinfectants by substituting them with natural disinfectants derived from sengon extract (*Falcataria moluccana*) and liquid smoke from bamboo stems, which are more effective in preventing microbial proliferation. The purpose of mixing the two ingredients is to determine the effectiveness of the two ingredients in inhibiting the growth of basic microbes from previous research, whether it will be better or not.

METHODS

This study was performed in the Microbiology Laboratory of the Rajawali Bandung Health Institute from February to May 2023. This study aimed to assess the efficacy of a combination of natural substances, specifically sengon twig wood extract with ethyl acetate solvent and bamboo stem liquid smoke, in preventing microbial development. This study employed an experimental methodology. The subjects of this investigation were *Salmonella typhimurium* ATCC 25241, *Candida albicans* ATCC 10231, *Aspergillus flavus* ATCC 9643, and microorganisms present on furniture. The sample utilized in this study comprised a blend of sengon twig wood extract with ethyl acetate solvent and bamboo stem liquid smoke. Liquid smoke is obtained by distillation for 6 hours at a temperature of 130-145◦c. While sengon twig wood extract is obtained through the macerization technique (pragita et al, 2020). The observed properties of the combination of ethyl acetate extract from sengon wood (*Falcataria moluccana*) and liquid smoke derived from bamboo stems (*Bambusa* sp).

Prior to conducting the inhibition test, it is essential to assess the fungal growth curve to identify the ideal or logarithmic phase. The method used to measure the growth curve in this research is uv-vis spectrophotometry. The growth curve of bacteria and fungus is assessed utilizing a UV-Vis Spectrophotometer at a wavelength of 600 nm. The best wavelength for measuring the turbidity of microbiological suspensions ranging from yellow to brown is identified as this wavelength (Listiani et al. 2021).

The antimicrobial inhibitory assay against bacteria and fungi was conducted by combining sengon twig wood extract, ethyl acetate solvent, and bamboo stem liquid smoke in various solution ratios: 50:50, 40:60, 30:70, 20:80, and 10:90. Mixing is done based on previous trials. The bacterial inhibition test was conducted utilizing the disc diffusion method on Nutrient Agar (NA) media, whilst the fungal inhibition test was performed using the well diffusion method on Potato Dextrose Agar (PDA) media (Pragita et al. 2020). Prior to conducting the inhibition test, it is essential to assess the fungal growth curve to identify the ideal or logarithmic phase. The test bacteria and fungi were inoculated into Nutrient Agar and Potato Dextrose Agar medium. A disc of paper, saturated with a blend of natural antibacterial disinfectants, was positioned atop the PDA media to create a well. The well was subsequently treated with a mixture of natural disinfectants. The incubation process was subsequently conducted using the optimal duration determined from the previously measured growth curve to see the formation of the inhibition zone (Pah et al. 2022).

Effectiveness testing was conducted by swabbing furniture, specifically a table, that had been treated with a natural disinfectant. The disinfectant applied was based on a volume comparison derived from the inhibition test results exhibiting the biggest inhibition zone. The testing method employed was the pour plate technique (Pah et al. 2022). The suspension derived from the swab findings right on the table was subsequently dissolved in NaCl, inoculated onto NA and PDA media, and incubated for the optimal growth durations of 24 hours for bacteria and 72 hours for fungus. Subsequently, the proliferation of bacteria and fungi was observed (Pah et al. 2022). Data taken from research results conducted in the microbiology laboratory of rajawali health institute bandung and based on colony growth appearing on test media. Data processing was done using microsoft excel, which has previously been calculated the number of colonial counts. The data was then calculated using the percent formula to be then presented in the form of a diagram.

RESULTS AND DISCUSSION

Identification of Test Microorganisms

The observation results in the table above are in accordance with research conducted by Amirudin in 2017 which stated that salmonella colonies are round, have a smooth texture, convex elevation and are shiny (Amiruddin et al. 2017). Research by Praja et al. (2017) indicates that Aspergillus flavus initially appears white, subsequently transitioning to yellowish-green, then yellow, and finally brown on the bottom surface (Praja & Yudhana, 2017). Research by Pah et al. (2022) similarly demonstrated that *Candida albicans* exhibits round to oval colonies characterized by a white to yellowish coloration, convex elevation, smooth and uniform cell margins, and a distinctive yeast-like odor (Pah et al. 2022).

Table 1. Macroscopic identification results of test microbes

Type of microbe	Macroscopic Image	Observation
S.typhimurium		The colony has a convex elevation, is white in color and is round in shape
A.flavus		The colonies appear greenish yellow, shaped like sand
C.albicans		Colonies exhibit a round or oval morphology, protruding slightly above the surface of the medium. The coloration ranges from white to yellowish white, accompanied by a characteristic yeast cell aroma and a faintly sour scent

Growth Curve

During the logarithmic phase, cells proliferate at a consistent rate, mass increases proportionately, metabolic activity is stable, and growth is equilibrated (Pah et al. 2022). The logarithmic phase test results indicated that Salmonella typhimurium ATCC 25241 exhibited logarithmic growth between 4-28 hours, Aspergillus flavus ATCC 9643 between 21-40 hours, and Candida albicans ATCC 10231 between 16-44 hours. During this phase, the proliferation and division of microorganisms reach their peak, resulting in the production of several primary metabolites, including proteins, lipids, carbohydrates, nucleotides, amino acids, and nucleic acids. The lag phase for *Salmonella typhimurium* ATCC 25241 occurred between 0-2 hours, for *Aspergillus flavus* ATCC 9643 between 0-20 hours, and for *Candida albicans* ATCC 10231 between 0-12 hours; this phase is marked by an increase in absorbance as measured by a spectrophotometer. The stationary phase for *Salmonella typhimurium* ATCC 25241 commenced at the 30th hour, for *Aspergillus flavus* ATCC 9643 at the 41st hour, and for *Candida albicans* ATCC 10231 at the 48th hour. This phase is characterized by the onset of a flat or stable graph, indicative of nutrient depletion from the medium. The outcomes of the growth curve assessments are presented in Table 1.

Table 2. The result of Levene test (homogenity)

	Phase (1st hour)			
Type of microbe	**Lag**	**Log**	**Stasioner**	**Optimum time**
S.typhimurium	0-2	4-8	30-34	28
C.albicans	0-12	16-44	48	44
A.flavus	0-20	24-40	44	40

The colony collection times for the inhibition test were as follows: *Salmonella typhimurium* ATCC 25241 at 28 hours, *Aspergillus flavus* ATCC 9643 at 40 hours, and *Candida albicans* ATCC 10231 at 44 hours. A study by Firdausia et al. (2022) determined that the optimal period for the inhibition test on *Salmonella typhi* was at 18 hours, whereas for *Candida albicans*, it was at 36 hours. According to research by Fitriani et al. (2022), the optimal duration for Aspergillus flavus was found to be between 16 and 40 hours.

Differences in growth curve results occur due to factors that can influence mushroom growth, namely humidity, temperature, acidity, substrate (pH) and chemical compounds in the environment (Fitriani et al. 2022). Every table must be numbered and include a title at the top.

Characteristics of the Mixture of Ethyl Acetate Extract of Sengon Twig Wood and Liquid Smoke from Bamboo Stems

The macroscopic examination of the ethyl acetate extract from sengon twig wood reveals a brown coloration. Liquid smoke from bamboo stems observed macroscopically has a pH of 3, is clear, has a pungent odor and is brownish yellow. The blackish brown color of liquid smoke is due to the tar content. The more transparent the liquid smoke, the better its quality. The lower the pH value of the liquid smoke, the higher the quality of the liquid smoke, this will affect the resulting inhibitory power. While the pungent odor is due to the presence of phenol compounds. The distinctive odor of liquid smoke, apart from the pungent and sharp phenol content, is also obtained from the presence of other compounds such as lactones and carbonyls (Lestari et al. 2015). The acrid scent of the combination of 11% ethyl acetate extract and 100% sengon twig wood liquid smoke arises from the phenolic compounds present in the liquid smoke, while the observed color variations in the comparisons result in progressively lighter hues due to the incremental addition of liquid smoke in each instance. The results of observations of the characteristics of ethyl acetate extract of sengon twig wood macroscopically can be seen in Table 2.

Table 3. Characteristics of the mixture of ethyl acetate extract of sengon twig wood and liquid smoke from bamboo stems

Comparison	Color	Turbidity	Smell	pH (Potential of Hydrogen)
50:50	Brown to orange	Cloudy	Sting	3
40:60	Brown, slightly orange	Cloudy	Sting	3
30:70	Brown	Cloudy	Sting	3
20:80	Light brown	Cloudy	Sting	3
10:90	Pale light brown	Cloudy	Sting	3

Effectiveness of the Mixture of Ethyl Acetate Extract of Sengon Twig Wood and Liquid Smoke from Bamboo Stems

Based on the results of the study on the effectiveness of a mixture of ethyl acetate extract of sengon twig wood and bamboo stem liquid acid with a ratio of 50:50, 40:60, 30:70, 20:80 and 10:90 against *Salmonella typhimurium* ATCC 245241, *Aspergillus flavus* ATCC 9643 and *Candida albicans* ATCC 10231 are as follows.

Table 4. Diameter of the inhibition zone of the mixture of ethyl acetate extract of sengon twig wood and liquid smoke from bamboo stems on test microbes

Type of microbe	Inhibition zone diameter in comparison				
	50:50	40:60	30:70	20:80	10:90
S. typhimurium ATCC 245241	11 mm	11,8 mm	8,5 mm	10,1 mm	6,6 mm
A. flavus ATCC 9643	2,5 mm	4 mm	5,5 mm	5 mm	0 mm
C. albicans ATCC 10231	3,8 mm	2,5 mm	5,3 mm	4,7 mm	3 mm

The results of the Salmonella typhimurium ATCC 25241 inhibition test indicate that the optimal mixture for inhibiting bacterial growth consists of 11% ethyl acetate extract of sengon twig wood and 100% bamboo stem liquid smoke at a volume ratio of 40:60, yielding an inhibition zone of 11.8 mm, which can be classified as a strong inhibition zone strength. In *Aspergillus flavus* ATCC 9643, the optimal volume ratio for growth inhibition is 30:70, yielding a moderate inhibition zone strength of 5.5 mm. In *Candida albicans* ATCC 10231, the largest inhibition zone, also at a ratio of 30:70, measures an average of 5.33 mm, which is similarly categorized as moderate inhibition. According to the provisions of the weak category of antimicrobial strength is in the range of <5 mm the diameter of the clear zone formed is a moderate category of 5-10 mm, the strong category is> 10-20 mm. and the category is very strong if the clear zone formed is > 20 mm (Jawetz et al. 2016).

The clear zone formed in the *C. albicans* ATCC 10231 culture using the well method is due to the mixture of sengon twig wood extract with ethyl acetate solvent containing flavonoid, tannin, saponin and steroid compounds (Pah et al. 2022). The antimicrobial mechanism of flavonoids involves the impairment of membranes and cell walls, while tannins inflict damage on proteins and disrupt the biosynthesis of cell walls and membranes. Saponins, in antifungal applications, can compromise fungal cell membranes and inhibit yeast cell proliferation, whereas steroids can also damage cell membranes (Balafif et al, 2017). Meanwhile, 100% bamboo stem liquid smoke has phenol compounds that can inhibit the growth of fungi which can be used as an antimicrobial (Pah et al. 2022).

Based on research conducted by Pragita et al. (2020), which tested the inhibitory power of ethyl acetate extract of sengon twig wood against *Salmonella typhi* with a concentration of 11%, namely 9.1 mm. In the research results of Abiyoga et al. (2021), the inhibition zone of 40% red betel leaf ethanol extract against *Aspergillus flavus* was around 20.62 mm. The results of research by Listiani (2021) on the antimicrobial activation test of ethyl acetate extract of sick sengon twig wood with a concentration of 11% on *C. albicans* obtained the results of the inhibition zone diameter of 3.7 mm and the results of research conducted by Pah et al. (2021) on the effectiveness test of 10% bamboo stem liquid smoke from pyrolysis, one of the test fungi was *C. albicans* ATTC 10231 which was carried out using two well and disc methods, the results showed that no inhibition zone of 0 mm was formed (Listiani et al. 2021). The difference in the size of the inhibition zone diameter can vary. This can be influenced by several factors, including agar thickness, pre-diffusion time, agar media composition, inoculum density, incubation time, incubation temperature, microbial species, pH, and antimicrobials contained in the disc or well (Pragita et al. 2020).

Effectiveness of a Mixture of Liquid Smoke and Sengon Branch Diseased Wood Extract with Ethyl Acetate Solvent as a Disinfectant

The number of colonies that grow on NA and PDA media is calculated to then see its effectiveness in inhibiting the growth of bacteria and fungi. Results The combination of ethyl acetate extract from sengon twig wood and liquid smoke derived from bamboo stems in ratio 40:60 demonstrates a 92% efficacy in inhibiting bacterial growth and a 73% efficacy in inhibiting fungal growth. In contrast, the positive control disinfectants based on carbolic acid exhibit a 100% efficacy against bacteria and an 86% efficacy against fungi. Additionally, ethyl acetate solvent alone inhibits bacterial growth by 85.4% and fungal growth by 60.5%. These results show that the combination of natural ingredients can inhibit microbial growth. This is because liquid smoke contains compounds that can inhibit microbial growth, according to Swastawati et al. (2017) in the writing of Pah et al. (2022) stated that these compounds are lignin, cellulose, and hemilulose. In addition, liquid smoke also contains phenol, carbonyl and acid (Erlytasari et al. 2019). This is reinforced by the extract of sengon twig sick wood which also has secondary metabolite compounds. The extract of sengon twig sick wood has febolic, flavonoid, saponin and tannin compounds which can inhibit microbial growth (Pragita et al. 2020). In addition, the solvent used to dissolve the extract, namely ethyl acetate, also can inhibit microbial growth (Hartati et al. 2017). The results of the effectiveness of the combination of ethyl acetate extract of sengon twig wood and liquid smoke from bamboo stems can be seen in Figure 1.

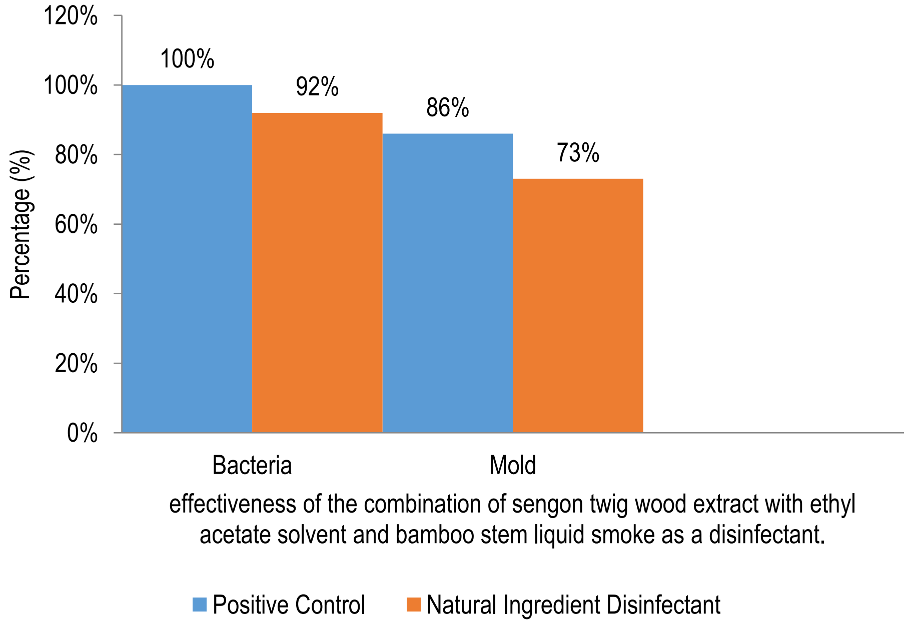

Figure 1. The results of the effectiveness of the combination of sengon twig wood extract with ethyl acetate solvent and bamboo stem liquid smoke as a disinfectant

The assessment of the characteristics of the combination of sengon twig wood extract with ethyl acetate solvent and bamboo stem liquid smoke was carried out by respondents when spraying on the table was carried out. The results of the questionnaire on the use of natural ingredient combination disinfectants can be seen in Figure 2.

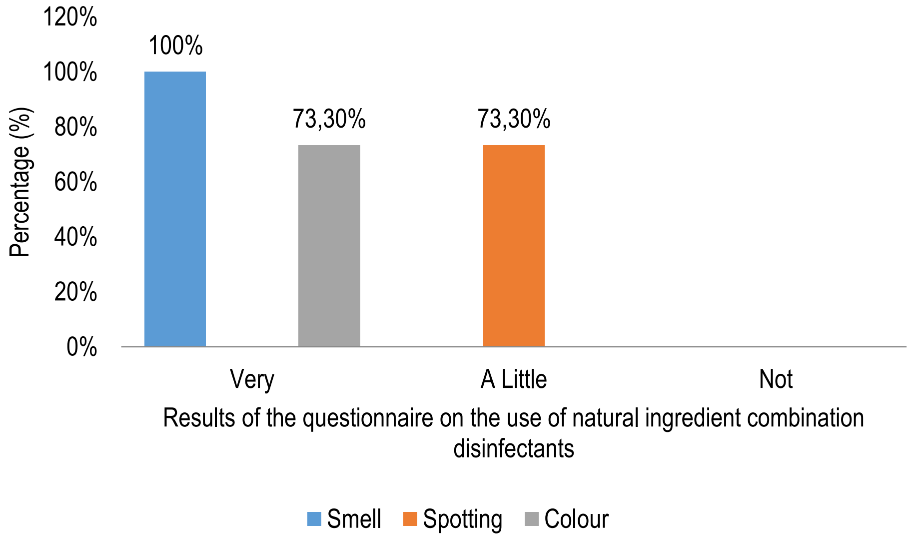

Figure 2. Results of the questionnaire on the use of natural ingredient combination disinfectants

To know the characteristics of natural combination disinfectants, we used a survey method on respondents. The volume of the combination that was assessed was 40 ml of sengon wood ethyl acetate extract and 60 ml of bamboo stem liquid smoke. Based on Figure 2. Characteristics of the combination of natural ingredients disinfectant according to respondents, as many as 100% said that the smell of the combination was pungent. With the remaining stains on the table, 73.3% of respondents said only a few stains were visible. As for color, 73.3% of respondents said that the color it has is very thick.

The limitation of this study is that phytochemical analysis has not been carried out to find out more about the compounds contained in the disinfectant material of a mixture of ethyl acetate extract of sengon twig wood 11% with 100% liquid smoke from bamboo stems at a volume ratio of 40:60. However, so far the use of combination of natural disinfectants with the purpose of minimizing the use or exposure to chemicals is recommended. It is hoped that further researchers can conduct further research on the compounds contained in a volume ratio of 40:60.

CONCLUSION

Based on the results of research that has been conducted both in vitro and in vivo, it can be concluded that the combination of ethyl acetate extract of sengon twig wood and liquid smoke from bamboo stems with a ratio of 40:60 has antibacterial and antifungal properties and is effective for use as a disinfectant.

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AUTHOR CONTRIBUTIONS

DA, RM, FRJ, DN: Data collection, analysis and interpretation of results, and drafting of the manuscript. FF, NSM: Concept and design of the study, analysis and interpretation of results, and finalization of the manuscript. AR: Concept and design of the study, analysis and interpretation of results, corresponding author, and finalization of the manuscript.

CONFLICTS OF INTEREST

Have no conflict of interest

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